Title:
Engineered *Saccharomyces boulardii* reduces colitis-associated colorectal cancer burden in a mouse model of inflammatory bowel disease

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Inflammatory bowel diseases affect over 1.5 million individuals in the United States and over 2 million individuals in Europe. Individuals with inflammatory bowel diseases have an increased risk of colorectal cancer driven by chronic inflammation. Current medical therapies include systemic infusions of antibodies targeting proinflammatory cytokines. The resulting immunosuppression increases the risk of opportunistic infections, among other side effects such as infusion reactions. Live biotherapeutics, including engineered probiotic organisms, are an emerging treatment modality that offers local rather than systemic treatment. This has the potential to limit systemic side effects. Our group has engineered an orally deliverable *Saccharomyces bouladrii* strain that not only expresses targeting ligands capable of binding to inflamed tissues but also possesses the capability to secrete anti-inflammatory molecules. In this study, we used a well-established model of IBD-associated colorectal cancer to test the efficacy of our engineered *S. boulardii.* Germ-free *Il10*$^{-/-}$ mice were colonized with a specific-pathogen free fecal microbiota transplant to initiate inflammation and then were injected with azoxymethane to induce colonic carcinogenesis. Mice were treated with engineered *S. boulardii* via oral gavage at two dosing frequencies (n=20 once weekly, n=17 twice weekly) versus placebo (n=18) for 16 weeks after colonization, until the end of the model. We hypothesized that engineered *S. boulardii* would reduce tumor burden versus placebo-treated controls. Stools were collected one week after colonization and at the time of harvest to non-invasively assess intestinal inflammation using fecal lipocalin (Lcn2) ELISA. Colonic tissues were collected at the time of harvest for gross, histological, and molecular analysis of inflammation and tumorigenesis. There were no differences in survival or body weight between groups. Fecal Lcn2 ELISA revealed that all groups developed intestinal inflammation over time. Engineered *S. boulardii* reduced gross tumor number in a dose dependent manner with median tumor counts equal to 7.5 in the placebo group, 5 in mice treated with engineered *S. boulardii* once weekly, and 3 in the group treated twice weekly (p=0.0014). Furthermore, distal colonic *Il12p40*, *Il23p19*, and *Tgfb* expression were decreased in a dose-dependent manner. Histological inflammation and tumor invasion were not different between placebo and once weekly *S. boulardii*. Histological scoring of twice weekly *S. boulardii* administration cohorts are in process. Together, our data suggest that engineered *S. boulardii* may reduce colorectal cancer tumor burden with associated changes in the inflammatory milieu.